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Pharmacokinetics of 1,25-dihydroxyvitamin D_3 glycosides from *Solanum glaucophyllum* extract given in a rumen bolus on blood mineral profiles in dry pregnant dairy cows

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ABSTRACT

Providing tablets of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the biologically active metabolite of vitamin D_3, in a rumen bolus may be used as prevention for periparturient hypocalcemia in dairy cows. This study investigated the pharmacokinetics of $1,25(OH)_2D_3$ glycosides extracted from *Solanum glaucophyllum* (SGE) on blood serum $1,25(OH)_2D_3$, Ca, P and Mg response in dry pregnant dairy cows. Boluses contained tablets of SGE which differed in their release properties (rapid release, slow release and combination) and galenics (200 µg uncoated, 300 µg and 500 µg uncoated or coated, 2 × 500 µg uncoated). Nineteen blood samples were collected from 29 cows between 96 h before and 336 h after bolus administration. Blood serum $1,25(OH)_2D_3$, Ca and P increased between 12 h and 120 h, 12 h and 264 h and 24 h and 264 h, respectively. Highest values were reached at 30 h, 72 h and 120 h for $1,25(OH)_2D_3$, Ca and P, respectively. Baseline values were then reached at 216 h for 1,25 $(OH)_2D_3$ and 336 h for Ca and P. Concentration of Mg decreased between 24 h and 216 h, before reaching values comparable to baseline at 264 h. Highest Ca values were obtained with the combined rapid and slow release properties (500 µg) and there was no effect from coating on pharmacokinetics. In conclusion, the antepartum oral SGE bolus administration may be suitable for the prevention of periparturient hypocalcemia.

1. Introduction

The sudden onset of milk production at calving time induces a drastic increase in calcium (Ca) requirements for dairy cows. In order to maintain a constant blood Ca level, the cow's homeostatic regulation mechanisms increase Ca resorption from body reserves in the bones and intestinal Ca absorption. Blood Ca levels are regulated by parathyroid hormone (PTH), calcitonin and 1,25-dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$), the biologically active metabolite of vitamin D_3. The physiological range of blood serum Ca concentration is between 2.0 and 2.8 mmol/l; the range between 1.4 and 2.0 mmol/l is considered to correspond to subclinical hypocalcemia; levels <1.4 mmol/l are associated with clinical hypocalcemia, i.e. with a high risk for milk fever occurrence (Braun et al., 2009; DeGaris and Lean, 2009). Reported incidence of puerperal clinical hypocalcemia in dairy cows varies between 1% and 10% (Martin-Tereso and Martens, 2014; DeGaris and Lean, 2009). These values depend on parity as primiparous cows are less susceptible than multiparous cows, especially from the 3rd lactation onwards (Caixeta et al., 2015; Goff, 2014; Oetzel, 2011; Reinhardt et al., 2011). An incidence of subclinical hypocalcemia of 50% or more has been reported for older cows (Caixeta et al., 2015; Reinhardt et al., 2011; Venjakob et al., 2017). Low postpartum blood Ca levels can predispose to various puerperal diseases such as dystocia, uterus prolapse, placental retention, metritis, mastitis, abomasum displacement and ketosis (Mulligan et al., 2006; Rodriguez et al., 2017; Seifi and Kia, 2018). Strategies to limit the risk of milk fever occurrence include: a diet low in Ca (Thilsing-Hansen et al., 2002; Roche et al., 2018; Kerwin et al., 2019); presenting a negative dietary cation-anion balance during the last three to four weeks prepartum (Goff, 2014; Martin-Tereso and Martens, 2014; Lean et al., 2019; Santos et al., 2019); oral (Hove and Kristiansen, 1984) or parenteral administration of vitamin D_3 (injections) a few days before (Goff, 2014; Ishii et al., 2015; Starič and Hodnik, 2021) or immediately after calving (Vieira-Neto et al., 2017; Vieira-Neto et al., 2021); as well as parenteral (Wilms et al., 2019; Amanlou et al., 2016; Miltenburg et al.,

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2016) or oral administration of soluble forms of Ca around parturition (Goff, 2008; Thilsing-Hansen et al., 2002) are recommended as well, in the case the two main dietary strategies would be insufficient.

In a natural environment with sufficient ultraviolet B light, ruminants can fulfill most of their vitamin D_3 requirements through synthesis in addition to vitamin D_3 ingestion over feed sources (Hymøller and Jensen, 2010). The de-novo synthesis of vitamin D_3 involves splitting of the cholesterol steroid ring by ultraviolet B light in the skin. Vitamin D_3 is first hydroxylated in the liver to 25-hydroxyvitamin D_3 ($25(OH)D_3$). A second hydroxylation triggered among others by PTH, which is secreted by the parathyroid gland in response to low blood Ca concentration, results in the synthesis of the active metabolite $1,25(OH)_2D_3$ in the kidneys (DeGaris and Lean, 2009; Goff, 2014). Active intestinal Ca uptake to the blood compartment, aside from passive absorption through paracellular transport, is promoted by increased expression of Ca channels in the apical membrane of intestinal epithelial cells, of calbindins (Ca binding proteins for intracellular Ca transport), and of extrusion systems (for Ca release from the basolateral membrane) under the influence of $1,25(OH)_2D_3$ (Goff, 2014; Martin-Tereso and Martens, 2014; van de Graaf et al., 2006).

Therefore, oral administration of the biologically active metabolite $1,25(OH)_2D_3$ may be an efficient strategy to prevent hypocalcemia in periparturient cows. The plant *Solanum glaucophyllum* contains 1,25 $(OH)_2D_3$ in the form of glycosides. The glycosides are cleaved by rumen bacteria and free $1,25(OH)_2D_3$ is released after ingestion (Napoli et al., 1977; Horst et al., 2003). Feeding dried leaves of *S. glaucophyllum* to cows starting two weeks before parturition permitted to maintain slightly higher blood serum Ca levels at and in the first days after calving (Horst et al., 2003; Ishii et al., 2015). However, correct timing and slow withdrawal of the exogenous source of $1,25(OH)_2D_3$ are crucial when using external sources of $1,25(OH)_2D_3$. Due to the potential of a reduced endogenous $1,25(OH)_2D_3$ production in these cases, sudden withdrawal can lead to a rebound effect which can trigger late milk fever (Horst et al., 2003; Goff, 2008).

Concentrated $1,25(OH)_2D_3$ glycosides can be extracted from *S. glaucophyllum*, allowing for the development of products which should be easier to manage than leaves and easier to apply to cows for the prevention of hypocalcemia and milk fever. Tablets of $1,25(OH)_2D_3$ glycosides extracted from *S. glaucophyllum* (SGE) were developed to enable a combination of rapid and long-acting slow release of 1,25 $(OH)_2D_3$ in the rumen to provide a suitable window of application with an optimal release pattern for the prevention of hypocalcemia in dairy cows. These SGE tablets can then be incorporated in a single ruminal bolus to be applied prior to calving.

The aim of this study was to investigate the pharmacokinetics of 1,25 $(OH)_2D_3$ and the response in Ca, P and Mg blood concentrations in dry pregnant dairy cows following the administration of oral boluses containing SGE tablets differing in their release properties (rapid, slow) and galenics (uncoated, coated). This, to determine the influence of the release properties and the galenic form on the pharmacokinetics and responses and the optimal administration time prior to calving of the bolus combining SGE tablets with the two release properties.

2. Materials and methods

2.1. Animals and housing

Twenty-nine healthy, dry pregnant Holstein and Red Holstein dairy cows (220 to 257 d after successful insemination) from the Agroscope dairy herd (Posieux, Switzerland) were allocated to five successive groups according to parity (primiparous, multiparous) and expected calving date. One group consisted of six primiparous cows, and four groups of multiparous cows (2^{nd} to 6^{th} parity, three groups with six cows and one group with five cows). Each cow within a group was randomly allocated to one of six treatments, whereby during the last two multiparous groups, cows were allocated in order that parity numbers were evenly distributed between treatments.

The cows were housed in a tie-stall barn with rubber mat flooring during the entire experimental period, which lasted for 14 d per animal. The cows were adapted to the diet for 10 to 13 d prior start of the experiment and a veterinarian performed a complete physical examination of each cow. Each cow was weighted 4 days prior start and at the end of the experiment. After completion of the experiment, the cows were housed in individual boxes with deep straw until calving. The course of parturition was recorded and the cows were returned to the free stall barn a few days after calving. All procedures were conducted in accordance with the Ordinance on Animal Protection and the Ordinance on Animal Experimentation. The experiment was approved by the competent authorities (Authorization 2017_24_FR).

2.2. Diet

The cows had free access to water and hay (2nd seasonal harvest; growth development stage "begin heading" according to *Dactylis glomerata*; mixed botanical composition with 78% graminea, 19% legumes, 3% other plants) and were fed daily 300 g of pelleted mineralized concentrate. This concentrate was formulated to meet or exceed the Swiss feeding recommendations for dry and pregnant cow (Agroscope, 2017) and consisted of wheat bran, apple pomace, oats, sodium chloride, magnesium oxide, molasses, trace minerals and vitamin premix. The trace mineral and vitamin premix contained 676′000 IU vitamin D_3 per kg and was included in the mineralized concentrate at 1.6%. Daily hay intake was calculated twice as the difference in weight between offered and refused amounts on three consecutive days (d3 to d5 and d10 to d12). Individual water consumption was recorded daily.

2.3. Treatments

The $1,25(OH)_2D_3$ provided in form of glycosides was extracted from *S. glaucophyllum* leaves and compounded into galenic tablet formulations (Herbonis Animal Health GmbH, Augst, Switzerland). The rapid release (RR) tablets were uncoated (u) and the slow release (SR) tablets were either coated (c) or not (u). The tablets were filled into gelatin-based capsules with dolomite and $CaMg(CO_3)_2$ as ballast to produce bolus formulations containing a total of 200, 300 and 500 μg 1,25 $(OH)_2D_3$ from RR, SR and a combination of RR and SR tablets, respectively. The bolus with 500 μg $1,25(OH)_2D_3$ equals the sum of the SR and RR tablets from boluses containing 200 and 300 μg $1,25(OH)_2D_3$, respectively. The bolus with 500 μg $1,25(OH)_2D_3$ was studied previously (Bachmann et al., 2017), but with different release properties and galenics.

The six treatments consisted of the oral administration of one 200u (RR only), 300u (SR only, uncoated), 500u (combination of RR and SR, uncoated), 300c (SR only, coated) and 500c (combination of RR and SR, coated) bolus and the simultaneous oral administration of two 500u boluses (total 1000 μg $1,25(OH)_2D_3$, 2x500u).

The bolus was applied on d0 to each cow per group using a bolus applicator (V-Grip™ Bolus Gun, item 64,314, MAI Animal Health, Melksham, Wiltshire UK). The treatment 2x500u was applied to five cows (vs. six for the other treatments), as one animal assigned to this treatment dropped out prior to the start of the study and could not be replaced, because no suitable dry and pregnant cow (220–257 days after successful insemination) was available at that time.

2.4. Sample collection and preparation

Nineteen blood samples were taken from each cow at -96 h, -72 h, 0 h, 1 h, 3 h, 6 h, 12 h, 18 h, 24 h, 27 h, 30 h, 48 h, 72 h, 96 h around bolus application, as well as at 120 h (d5), 168 h (d7), 216 h (d9), 264 h (d11) and 336 h (d14) after treatment. Sample of 0 h was taken on d0 at 08 h 00 immediately prior to bolus application. Within a group and sampling time, blood was collected within 30 min, always in the same order of

cows. Blood was sampled from the jugular vein, alternatively on the right and left side, using disposable cannulae (Vacuette 20G, Greiner Bio One International GmbH, Solingen, Germany) and serum vacutainers without anticoagulant (S-Monovette, Sarstedt AG + Co. KG, Nümbrecht, Germany). Serum vacutainers were centrifuged within 2 h of sampling at 1500 x *g* for 15 min followed by 4000 x g for 2 min. The serum was immediately separated and stored at −20 °C until analyses were performed. Hay and concentrate samples were collected twice a week and pooled over two-week periods. Pooled hay and pooled concentrate samples were milled separately (1.0 mm, Brabender mill, Duisburg, Germany) and preserved in glass bottles until analysis. One bolus of each formulation was randomly selected and the tablets were milled after extraction from the gelatin capsules and preserved in glass bottles until analysis.

2.5. Chemical analyses

Blood serum concentration (0 h, 12 h, 30 h, 48 h, 72 h, 120 h and 216 h) and SGE Bolus content of $1,25(OH)_2D_3$ were determined using ELISA kits (Kit 2112, Immundiagnostik, Bensheim, Germany). Concentrations of blood serum Ca, P and Mg (−96 h to 336 h) were determined in duplicates colorimetrically, for Ca with an autoanalyzer Cobas Mira (Roche, Basel, Switzerland), and for P and Mg using commercial kits with an autoanalyzer Alizé 200 (BioMérieux, Marcy l'Etoile, France). Dry matter (DM), ash, crude fiber (CF), crude protein (CP) and mineral contents (Ca, P, Mg, K and Na), respectively in hay and mineralized concentrate, were determined as described by Oberson et al. (2019).

2.6. Statistical analyses

The individual cow was considered as the subject unit of the experiment and acted as its own control, based on initial baseline values. Initial baseline values for repeated blood analyses were calculated as a mean of values from −96 h, −72 h and 0 h. No control group needed to be added to the defined six groups, as blood serum Ca concentrations remain constant during the weeks prior to calving (Kimura et al., 2006; Rérat and Schlegel, 2014; Starič and Hodnik, 2021). Peak observed drug concentration (C_{max}), time of peak concentration (T_{max}), area under the concentration-time curve (AUC) and residence time for serum $1,25(OH)_2D_3$, Ca, P and Mg were calculated for each cow by non-compartmental analysis of the orally administered $1,25(OH)_2D_3$. This was done using R software (version 3.5.3, with NonCompart, ncar, dplyr and nlme packages) including the selected conditions of extravascular route, trapezoidal rule for measurement of AUC with linear-up linear-down for calculation and best fit for the terminal slope determination.

Non-repeated animal performance data (hay and water intake and body weight) as well as C_{max}, T_{max}, AUC and residence time of blood serum values were analyzed using the general linear model of SYSTAT 13 (SYSTAT Software Inc.) following the model $Y_{ij} = \mu + Group_i + Treatment_j + \varepsilon_{ij}$ where Y_{ij} is the response, μ the least-squares mean, $group_i$ the fixed effect of the group (i = 1–5), $treatment_j$ the fixed effect of the experimental treatment (j = 200u, 300u, 300c, 500u, 500c and 2x500u), and ε_{ij} the random error. Repeated blood serum concentrations were analyzed using the mixed model of SYSTAT 13 (SYSTAT Software Inc.) following the model $Y_{ijklm} = \mu + Group_i + Treatment_j + Time_k + Interaction_l + Cow_m + \varepsilon_{ijklm}$ where Y_{ijklm} is the response, μ the least-squares mean, $group_i$ the fixed random effect of the group, $treatment_i$ the fixed effect of the experimental treatment, $time_k$ the fixed effect of blood sampling time (k = 0 h, 1 h, 3 h, 6 h, 12 h, 18 h, 24 h, 27 h, 30 h, 48 h, 72 h, 96 h, 120 h, 168 h, 216 h, 264 h and 336 h), $interaction_l$ the interaction of $treatment_i$ and $time_k$, Cow_m the fixed random effect of the cow and ε_{ijklm} the random error. Differences between least square means were considered as significant at $P \leq 0.05$ and tendencies were noted at $P < 0.10$. Comparisons among least square means and between application of uncoated and coated tablets (300u and 500u vs. 300c and 500c) were calculated using Tukey's contrasts.

3. Results

3.1. Animals, diet and feed intake

The analyzed boluses had a $1,25(OH)_2D_3$ content of 198, 272, 491, 292 and 517 µg, for 200u, 300u, 500u, 300c and 500c, respectively. All cows tolerated oral bolus application well and remained healthy during the experiment and the observational post experimental period which ended two days after calving. No adverse effects were observed.

The $1,25(OH)_2D_3$ content in each bolus formulation was consistent with the formulated values. The nutrient concentration (mean ± SD) in the consumed hay (n = 4) was, per kg DM, 4.9 MJ net energy for lactation, 287 ± 6 g CF, 149 ± 4 g CP, 99 ± 18 g ash, 6.2 ± 0.4 g Ca, 4.4 ± 0.1 g P, 1.8 ± 0.1 g Mg, 39 ± 0.7 g K and 0.6 ± 0.1 g Na. The nutrient concentration in the consumed mineralized concentrate (n = 4) was, per kg DM, 118 ± 1 g CF, 119 ± 3 g CP, 181 ± 1 g ash, 7.8 ± 0.8 g Ca, 6.0 ± 0.2 g P, 22.0 ± 0.6 g Mg, 8.5 ± 0.3 g K and 38.5 ± 2.3 g Na. Hay intake (12.5 ± 0.7 kg DM/d, least square mean ± SEM), water intake (78.9 ± 6.4 l/d) and body weight (737 ± 31 kg at d-4; 747 ± 33 kg at d14) did not differ ($P > 0.10$) among treatments.

The cows calved 25 ± 6 d after the last blood sampling, with a parity of 3.0 ± 0.7 without differences ($P > 0.10$) among treatments. The 29 calves (12 females and 17 males) were healthy.

3.2. Pharmacokinetics of $1,25(OH)_2D_3$ after oral bolus application

Following oral bolus application, $1,25(OH)_2D_3$ blood serum concentration (Fig. 1; Supplementary Table 1) increased ($P < 0.001$), compared to baseline (30 pmol/l), within the timeframe of 12 h (149 pmol/l) to 120 h (92 pmol/l) with maximal values observed from 30 h (207 pmol/l) to 48 h (187 pmol/l). Blood serum $1,25(OH)_2D_3$ returned to baseline at 216 h, therefore analyses for $1,25(OH)_2D_3$ was discontinued after that sampling time. The mean increase in blood serum $1,25(OH)_2D_3$ from the baseline was 513% between 12 h and 120 h after bolus application. The time dependent response was more pronounced in 300u, 500u, 500c and 2x500u than in 200u and 300c (time x treatment interaction, $P < 0.05$). Overall, blood serum $1,25(OH)_2D_3$ tended ($P = 0.08$) to be higher in 500u and 500c than 200u and contrast analysis revealed that blood serum $1,25(OH)_2D_3$ did not differ ($P > 0.10$) between uncoated and coated formulations. No differences ($P > 0.10$) among treatments were observed for T_{max} and residence time of serum $1,25(OH)_2D_3$, but C_{max} of 500u was twice as high compared to 200u ($P = 0.09$) and AUC of 500u and 500c were twice as high compared to 200u ($P = 0.08$; Table 1).

3.3. Changes in Ca, P and Mg blood levels

Following SGE bolus application, blood serum Ca concentration (Fig. 2; Supplementary Table 2) increased ($P < 0.001$) from the baseline (2.23 mmol/l) within the timeframe of 12 h (2.36 mmol/l) to 264 h (2.49 mmol/l), with maximal values observed from 48 h (2.72 mmol/l) to 96 h (2.69 mmol/l), before returning to values comparable ($P > 0.10$) to the baseline at 336 h (2.25 mmol/l). The mean increase in blood serum Ca from the baseline was 16% between 12 h and 264 h after bolus application. Between 0 h and 336 h, the blood serum Ca of 300c was 4% lower ($P < 0.001$) than of 500c and 2x500u. There was no interaction of time x treatment ($P > 0.10$). Contrast analysis revealed that blood serum Ca response did not differ ($P > 0.10$) between uncoated and coated formulations. No differences among treatments were observed for T_{max} and residence time of blood serum Ca, but C_{max} was higher ($P < 0.05$) with 2x500u compared to 300c, and AUC was higher ($P < 0.05$) with 2x500u compared to 200u and 300c (Table 1).

Similarly, blood serum P concentration (Fig. 3; Supplementary Table 3) increased after SGE bolus application ($P < 0.001$) from the baseline (1.50 mmol/l) within the timeframe of 24 h (1.94 mmol/l) to 264 h (2.00 mmol/l), with maximal values observed from 72 h (2.55

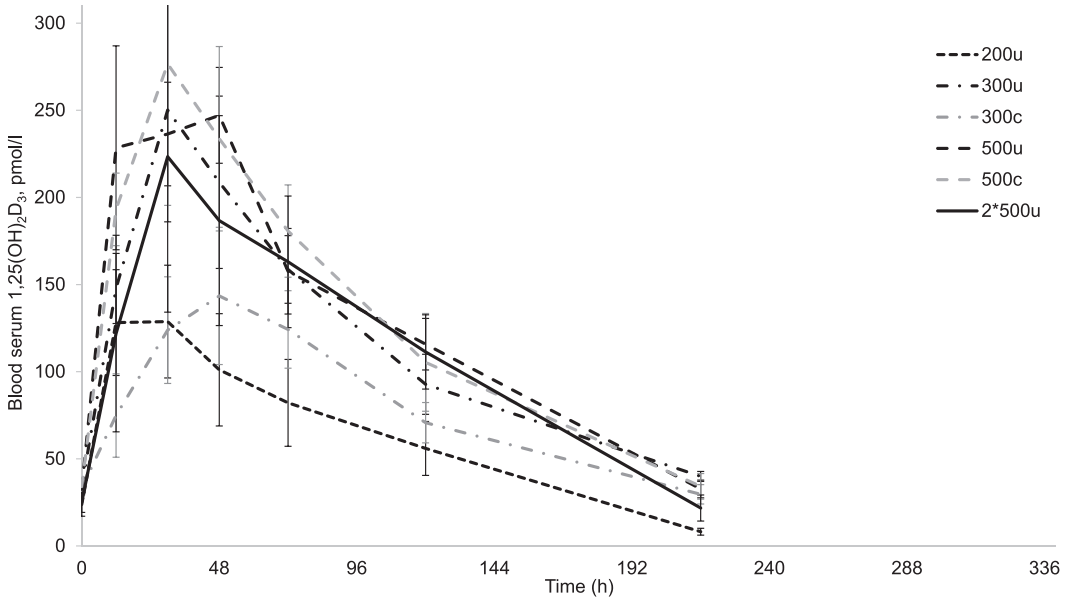

Fig. 1. Pharmacokinetics of $1,25(OH)_2D_3$ in blood serum (least square means ± SE) over time after single oral bolus administration (time effect: $P < 0.001$; treatment effect: $P < 0.001$; time x treatment interaction: $P > 0.10$).

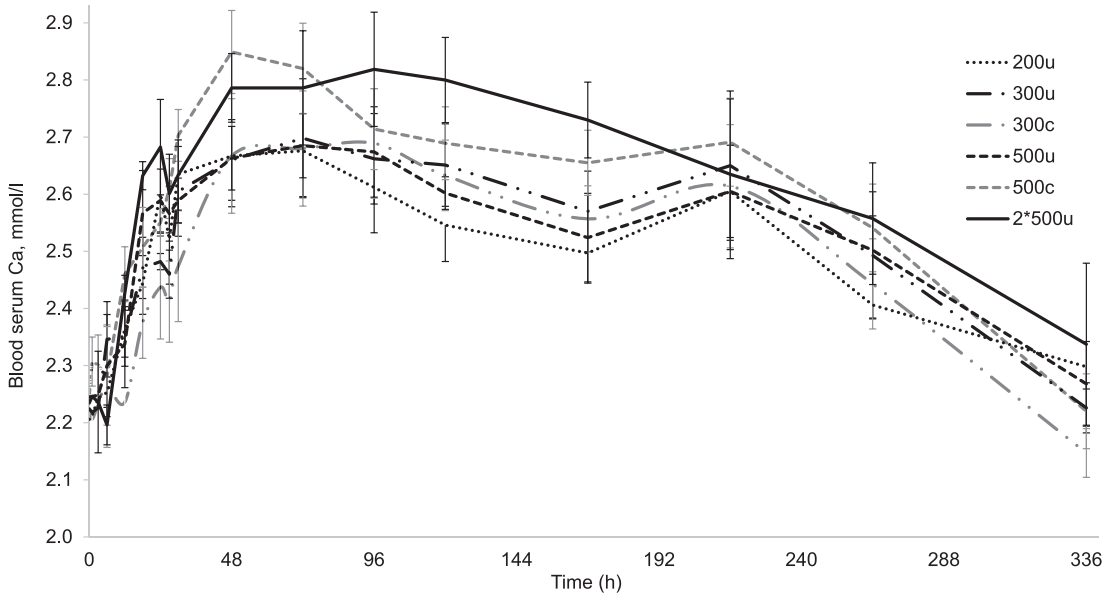

Fig. 2. Pharmacokinetics of calcium in blood serum (least square means ± SE) over time after single oral bolus administration (time effect: $P < 0.001$; treatment effect: $P < 0.001$; time x treatment interaction: $P > 0.10$).

mmol/l) to 120 h (2.73 mmol/l), before returning to values comparable ($P > 0.10$) to the baseline at 336 h (1.54 mmol/l). The mean increase in blood serum P from the baseline was 55% between 24 h and 264 h after bolus application. The response of blood serum P was similar between treatments ($P > 0.10$) and there was no time x treatment interaction ($P > 0.10$). Contrast analysis revealed that blood serum P response did not differ ($P > 0.10$) between uncoated and coated formulations. No differences among treatments were observed for C_{max}, T_{max}, AUC and residence time of serum P (Table 1).

Blood serum Mg concentration (Fig. 4; Supplementary Table 4) decreased ($P < 0.001$) from the baseline (0.91 mmol/l) within the timeframe of 24 h (0.83 mmol/l) to 216 h (0.82 mmol/l) with minimal values observed from 48 h (0.76 mmol/l) to 96 h (0.79 mmol/l), before reaching values comparable ($P > 0.10$) to the baseline at 264 h (0.86 mmol/l). The mean decrease in blood serum Mg from the baseline was 10% between 24 h and 216 h after bolus application. The response of blood serum Mg was similar between treatments ($P > 0.10$) and there was no time x treatment interaction ($P > 0.10$). Contrast analysis revealed that blood serum Mg response did not differ ($P > 0.10$) between uncoated and coated boluses. The highly variable blood serum Mg prevented to calculate accurate individual AUC and therefore the mean residence time.

4. Discussion

The oral application of boluses containing $1,25(OH)_2D_3$ glycosides

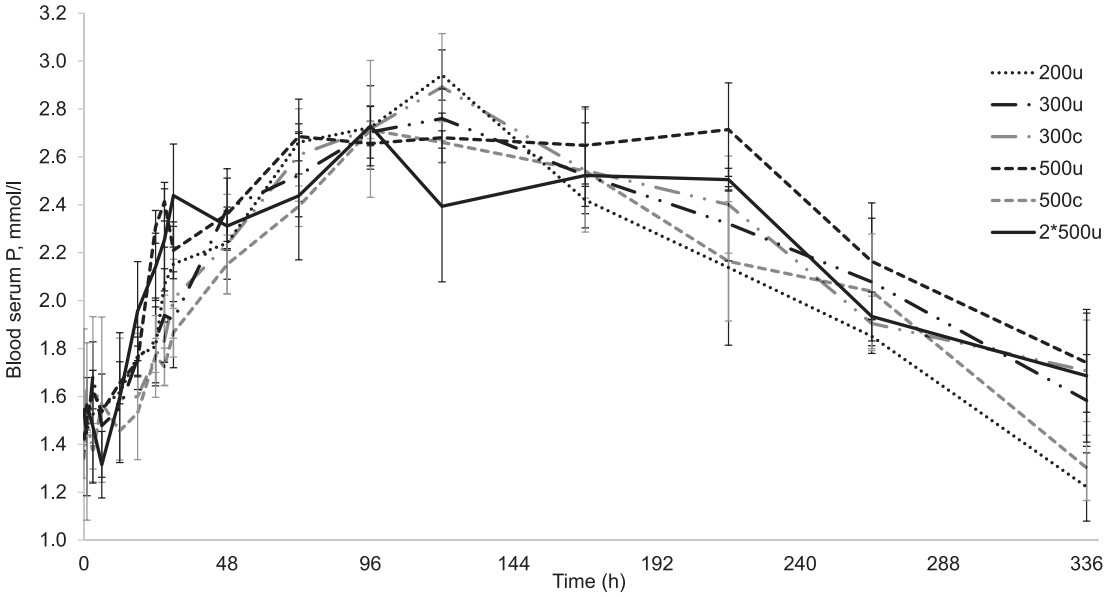

Fig. 3. Pharmacokinetics of phosphorus in blood serum (least square means ± SE) over time after single oral bolus administration (time effect: $P < 0.01$; treatment effect: $P < 0.001$; time x treatment interaction: $P > 0.10$).

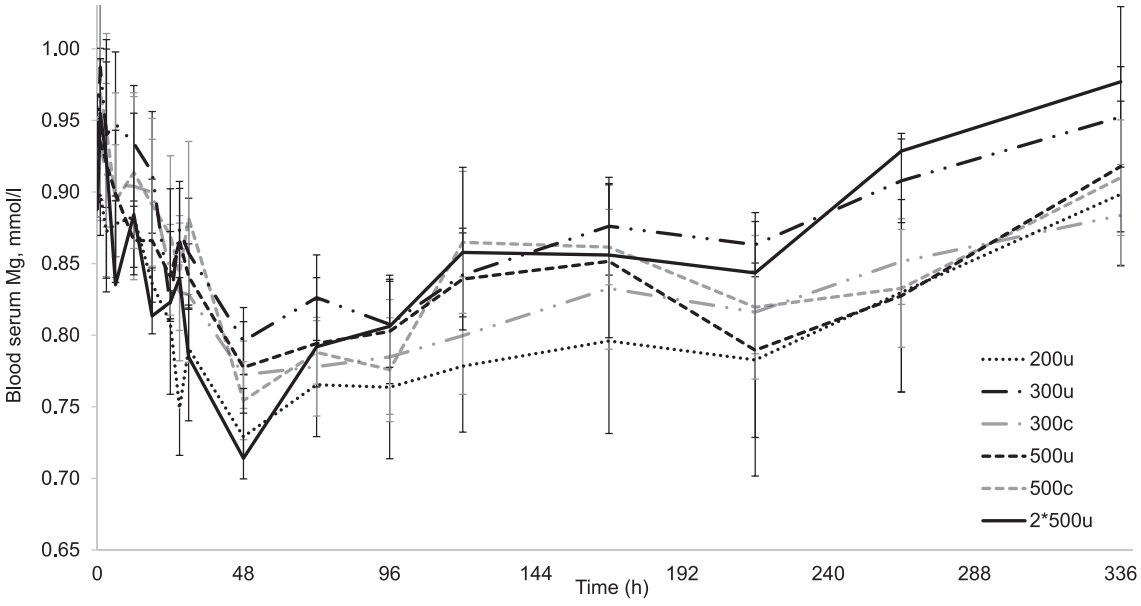

Fig. 4. Pharmacokinetics of magnesium in blood serum (least square means ± SE) over time after single oral bolus administration (time effect: $P < 0.001$; treatment effect: $P < 0.001$; time x treatment interaction: $P > 0.10$).

from *S. glaucophyllum* induced an increased blood serum $1,25(OH)_2D_3$ concentration. Subsequently, increased serum Ca and P concentrations and decreased serum Mg concentrations were observed in all treatments. This confirms the pharmacodynamical effect of SGE after oral administration as a bolus in pregnant dry cows on blood serum Ca, P and Mg concentrations. These results are in accordance with previous reports by others who fed the leaves of this plant (Roux et al., 1979; Horst et al., 2003; Ishii et al., 2015) or their extracted $1,25(OH)_2D_3$ glycosides (Bachmann et al., 2017).

Blood serum $1,25(OH)_2D_3$ concentrations between 12 and 48 pmol/ml have been reported in non-pregnant non-lactating cows, that rise to 48–120 pmol/ml in late pregnancy and to 240 pmol/ml around the time of parturition and onset of lactation. Elevated values up to >720 pmol/ml can be observed during severe hypocalcemia (Reinhardt et al., 1988). In the present study, the values observed prior to bolus administration in cows with advanced pregnancy, but not yet close to parturition were within these ranges and raised significantly from 12 h following bolus administration. However, the observed serum $1,25(OH)_2D_3$ concentrations did not exceed those reported in cows at the time of calving or in response to hypocalcemia after any treatment.

The mean serum Ca values prior to bolus application were within the normal range of 2.0–2.8 mmol/l. As Ca levels several weeks prior to parturition (220 to 257 days after successful insemination) can be assumed to be stable, the observed increase in Ca serum concentrations after bolus application solely originated from the orally applied product. The effect of preventive measures against hypocalcemia is meant to occur within 48 h after calving in order to limit the risk of milk fever (Goff, 2008; Venjakob et al., 2017). The maximal observed serum Ca

Table 1
Blood serum pharmacokinetics of $1,25(OH)_2D_3$, calcium and phosphorus.

		Treatment							
	Parameter, unit	200u	300u	300c	500u	500c	2x500u	SEM	*P*-value
$1,25(OH)_2D_3$	C_{max}, pmol/l	137	265	148	295	279	238	45.9	0.09
	T_{max}, h	19	42	44	37	37	36	7.4	n.s.
	AUC, pmol/l x min	14,309	25,611	17,547	28,643	28,659	25,202	1,622	0.08
	Residence time, h	72	79	85	76	73	83	3.6	n.s.
Ca	C_{max}, mmol/l	2.79^{ab}	2.79^{ab}	2.77^{b}	2.79^{ab}	2.89^{ab}	2.94^{a}	0.035	<0.05
	T_{max}, h	96	149	139	110	82	61	24.9	n.s.
	AUC, mmol/l x min	842^{b}	856^{ab}	844^{b}	853^{ab}	876^{ab}	884^{a}	9.0	<0.05
	Residence time, h	165	165	165	165	164	164	0.9	n.s.
P	C_{max}, mmol/l	3.03	2.81	2.89	3.01	2.75	2.83	0.123	n.s.
	T_{max}, h	134	120	139	139	115	71	24.6	n.s.
	AUC, mmol/l x min	727	757	760	799	722	756	44.5	n.s.
	Residence time, h	155	162	162	163	160	162	2.9	n.s.

Bolus with either uncoated (u) or coated (c) tablets totaling 200, 300 and 500 µg $1,25(OH)_2D_3$ provided as $1,25(OH)_2D_3$-glycosides from *Solanum glaucophyllum*.
[a,b]: values with different small letter superscripts differed significantly ($P \leq 0.05$) n.s.: non significant.
C_{max}: peak concentration of least square means.
T_{max}: time of peak concentration of least square means.
AUC: area under the concentration-time curve.

concentrations exceeded the normal range with 500c and 2x500u, only slightly and for a limited duration of 1–2 days. Therefore, adverse effects such as calcinosis should not be expected after single oral administration of one or two SGE boluses in the galenic forms used in the present study. Especially not during times of challenged Ca-metabolism as it is to be expected around calving. The observed plateau followed by a slow decrease in serum Ca concentrations lasted for 10 days. This long time-lapse should prevent delayed hypocalcemia observed in periparturient cows after abrupt withdrawal of feeding *S. glaucophyllum* leaves (Horst et al., 2003) and enlarges the window for the prevention of milk fever with a single application bolus.

The mean serum P values prior to bolus application were within the normal range of 1.4–2.6 mmol/l (Constable et al., 2017). The observed increase in P serum concentration after bolus application started later, but was more pronounced than that of Ca. Others (Bachmann et al., 2017; Ishii et al., 2015) have reported an increase in serum P concentrations after administration of $1,25(OH)_2D_3$ to cows outside the periparturient period, i.e. prior to the activation of Ca-mobilizing mechanisms such as increased PTH secretion, but these authors did not offer an explanation for this observation. Increased intestinal P absorption after supply of $1,25(OH)_2D_3$ has been reported in rodents (Marks et al., 2006) and in pigs (Fox and Care, 1979), but the mechanism (s) by which serum P concentrations increase in cows after treatment with $1,25(OH)_2D_3$ (and independently of PTH) remain unclear (Ishii et al., 2015). A positive association between serum Ca and P values has also been reported in parturient cows with and without clinically relevant hypocalcemia (Venjakob et al., 2017; Megahed et al., 2018). The observed decrease in serum P concentrations in those cows was attributed to increased P losses over colostrum and milk, as well as to increased renal P excretion under the influence of PTH secreted in response to hypocalcemia. The delayed serum P concentration increase compared to Ca observed here has also been reported by others (Bachmann et al., 2017; Ishii et al., 2015). A 12 h delay between the onset of increased Ca and P absorption was observed in isolated pig intestine perfused with a $1,25(OH)_2D_3$ solution, but not after perfusion with *S. glaucophyllum* extracts (Fox and Care, 1979). The reasons for these discrepancies and their mechanisms remain unknown.

The mean serum Mg values prior to bolus application were mostly within the normal range of 0.8–1.2 mmol/l (Martens, 2016). The observed decrease in serum Mg concentrations after treatment with $1,25(OH)_2D_3$ is in accordance with previous reports (Ishii et al., 2015; Littledike and Horst, 1982). This finding does not confirm the findings of Bachmann et al. (2017) who did not observe any effect of SGE on serum Mg concentrations. Magnesium is known to play an important role in the periparturient period as hypomagnesemia reduces PTH secretion as well as the tissue (bone, kidneys) response to PTH, and thus can precipitate the occurrence of milk fever (Goff, 2008, 2014; DeGaris and Lean, 2009). In contrast to the hormonal regulation of Ca and P via PTH and $1,25(OH)_2D_3$, Mg homeostasis is primarily regulated by dietary Mg intake and gastrointestinal absorption on one hand, and renal excretion on the other hand. However, increased renal reabsorption of Mg under the influence of PTH through modification of the renal threshold for Mg excretion has been suggested (Martin-Tereso and Martens, 2014; Martens, 2016). The decrease in serum Mg concentrations observed in the present study in dry pregnant cows outside the periparturient period may be a consequence of decreased PTH levels (and thus increased renal excretion of Mg) caused by the increase in serum Ca values after treatment with $1,25(OH)_2D_3$ glycosides from *S. glaucophyllum*. This could not be confirmed in the frame of the present study, as urinary Ca, P and Mg were not measured. This slight effect on serum Mg concentrations should not be of clinical relevance under the metabolic conditions of cows in the periparturient period (except in case of insufficient dietary Mg intake).

It has been known for decades that feeding leaves of *S. glaucophyllum* leads to an increase in serum Ca and P concentrations which can be used for the prevention of parturient hypocalcemia (Roux et al., 1979). Both dose and timing of treatment with $1,25(OH)_2D_3$ glycosides from *S. glaucophyllum* have been reported to be important for the prevention of hypocalcemia (Ishii et al., 2015; Bachmann et al., 2017). Although the effects of *S. glaucophyllum* on serum Ca concentrations have already been investigated by these authors, the aim of the present study was to describe the pharmacokinetics and the effects on blood serum $1,25(OH)_2D_3$, Ca, P and Mg concentrations of new formulations in a new application form. The new formulations achieved through combination of three types of $1,25(OH)_2D_3$ releasing tablets (rapid or slow release, coated or uncoated) were conditioned in a single oral bolus that should be easy to use in practice for the prevention of milk fever.

The present results confirm that a single oral SGE bolus administration leads to a rapid increase in serum $1,25(OH)_2D_3$ concentrations with maxima observed after 1.5 to 2 days and a return to pre-treatment values within 9 days. It also leads to a rapid increase in serum Ca concentrations (within 12 h) with maxima observed between 2 and 4 days and a progressive return to pre-treatment values within 11 days, which offers a relatively long window for treatment prior to calving. Timely ante partum application is the greatest challenge associated with $1,25(OH)_2D_3$ treatment to prevent milk fever (Goff and Horst, 1990; Goff, 2014; Perruchoud et al., 2017), thus a reasonably extended window of opportunity for treatment reduces the risk of too early or too late application of $1,25(OH)_2D_3$. The coated and uncoated properties of the SGE tablets resulted to similar blood serum Ca, P and Mg patterns and

this likely reflects that the slow release tablet formulation already reached the maximal release time without requiring any coating. The difference up to a factor five (200 vs. 1000 μg) in the administered 1,25 $(OH)_2D_3$ doses was not reflected in Ca, P and Mg serum variations of corresponding magnitude. While the rapid release tablets (200u) in most cases produced the most limited effects, the double application of the combined rapid and slow release tablets (2x500u) were not always associated with the highest response. An imperfect dose-response relationship after *S. glaucophyllum* application was also observed by Ishii et al. (2015) and Bachmann et al. (2017), although their experimental designs do not allow direct comparisons except for one experiment, where non-pregnant non-lactating cows were fed 0, 0.5, 1.0 and 2.0 mg/kg body weight of $1,25(OH)_2D_3$ from dried *S. glaucophyllum* leaves during 14 days. This resulted in increases of serum Ca and P concentrations parallel to the dosage of $1,25(OH)_2D_3$ (Ishii et al., 2015). In the present study, the agreement between rapid, slow and their combination, resulting to a certain dose-response was better for Ca than for P, and the highest serum Ca values were obtained with the 2x500u, in terms of C_{max} as well as of AUC. Treatment with two SGE boluses containing 500 μg of $1,25(OH)_2D_3$ each was not included as a therapeutic option, but to monitor the effects of erroneous (double) application. This treatment produced the highest elevation of serum Ca (not P) but did not lead to prolonged elevation of serum Ca above the normal range. In general, in no treatment were any adverse effects observed during the experiment or while the consecutive post-experimental observation period. These results suggest that the application of two instead of one bolus should not lead to toxicity in case of a dosing error in the field, especially as this treatment would be applied only once yearly around the time of parturition. Finally, the slow decrease in serum Ca concentration after the plateau phase should prevent delayed hypocalcemia (rebound effect) such as observed a week after withdrawal of *S. glaucophyllum* leaves fed from 6 days before until 14 days after parturition (Horst et al., 2003).

Finally, the application of uncoated 500 μg SGE bolus appears to be adequate for further investigations in pregnant cows around the time of parturition as a mean to reduce the risk of hypocalcemia occurrence. This, because the highest blood serum Ca levels were obtained with this bolus and because there were no differences between the coated and uncoated boluses. Indeed, the blood serum Ca concentration has recently been shown to be maintained within the normal range over the critical first two days of lactation in multiparous cows after the application of one 500 μg SGE bolus three to four days prior to expected calving (Meyer-Binzegger et al., 2021).

5. Conclusion

The bolus containing 1,25-dihydroxyvitamin D_3 glycoside extracts of *S. glaucophyllum* was successful in modifying blood serum $1,25(OH)_2D_3$, Ca, P and Mg concentrations in dry pregnant dairy cows over several days. The antepartum oral SGE bolus (independent of release properties) administration may be suitable for the prevention of periparturient hypocalcemia. The simultaneous application of two boluses with 500 μg $1,25(OH)_2D_3$, that may potentially happen erroneously in practice, did not show any adverse effect compared to one single bolus regarding dairy (Amanlou et al., 2016) cow health or the measured blood parameters.

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Declaration of Competing Interest

The co-author K. Bühler is affiliated to Herbonis Animal Health GmbH, company that developed and provided the boluses. Co-author K. Bühler was involved neither in data collection nor in data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2021.11.011.

References

Agroscope, 2017. Apports alimentaires recommandés pour les ruminants. Agroscope. www.agroscope.admin.ch (Accessed 01 october 2017).
Amanlou, H., Akbari, A.P., Farsuni, N.E., Silva-Del-Rio, N., 2016. Effects of subcutaneous calcium administration at calving on mineral status, health, and production of Holstein cows. J. Dairy Sci. 99, 9199–9210.
Bachmann, H., Lanz, M., Kehrle, S., Bittner, W., Toggenburger, A., Mathis, G.A., Rambeck, W., 2017. Effects of a sustained release formulation of 1,25-dihydroxyvitamin D3-glycosides for milk fever prevention on serum 1,25-dihydroxyvitamin D3, calcium and phosphorus in dairy cows. J. Steroid Biochem. Mol. Biol. 173, 301–307.
Braun, U., Zulliger, P., Liesegang, A., Bleul, U., Hässig, M., 2009. Effect of intravenous calcium borogluconate and sodium phosphate in cows with parturient paresis. Vet. Rec. 164, 296–299.
Caixeta, L.S., Ospina, P.A., Capel, M.B., Nydam, D.V., 2015. The association of subclinical hypocalcemia, negative energy balance and disease with bodyweight change during the first 30 days post-partum in dairy cows milked with automatic milking systems. Vet. J. 204, 150–156.
Constable, P.D., Hinchcliff, K.W., Done, S.H., Grünberg, W., 2017. Preceded. In: Radostits, O.M. (Ed.), Veterinary Medicine : A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats, 11th ed. Elsevier, St. Louis, USA, p. 2308.
DeGaris, P., Lean, I., 2009. Milk fever in dairy cows: a review of pathophysiology and control principles. Vet. J. 176, 58–69.
Fox, J., Care, A.D., 1979. Effects of hydroxylated derivatives of vitamin D3 and of aqueous extracts of Solanum malacoxylon on the absorption of calcium, phosphate, sodium, potassium and water from the jejunum of pigs. J. Endocrinol. 82, 417–424.
Goff, J.P., 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176, 50–57.
Goff, J.P., 2014. Calcium and magnesium disorders. Vet. Clin. North Am. Food Anim. Pract. 30, 359–381.
Goff, J.P., Horst, R.L., 1990. Effect of subcutaneously released 24F-1,25-dihydroxyvitamin D3 on incidence of parturient paresis in dairy cows. J. Dairy Sci. 73, 406–412.
Horst, R., Goff, J.P., Gill, S., Dallorso, M.E., Pawlak, E., 2003. Using *Solanum Glaucophyllum* as a source of 1,25-dihydroxyvitamin D to prevent hypocalcemia in dairy cows. Acta Vet. Scand. 44.
Hove, K., Kristiansen, T., 1984. Oral 1,25-dihydroxyvitamin D3 in prevention of Milk fever. Acta Vet. Scand. 25 (4), 510–525.
Hymøller, L., Jensen, S.K., 2010. Vitamin D3 synthesis in the entire skin surface of dairy cows despite hair coverage. J. Dairy Sci. 93, 2025–2029.
Ishii, J., Uramoto, A., Nagao, Y., Goto, H., 2015. Feeding *Solanum glaucophyllum* to preparturient multiparous cows prevents postparturient hypocalcemia. Anim. Sci. J. 86, 869–877.
Kerwin, A.L., Ryan, C.M., Leno, B.M., Jakobsen, M., Theilgaard, P., Barbano, D.M., Overton, T.R., 2019. Effects of feeding synthetic zeolite a during the prepartum period on serum mineral concentration, oxidant status, and performance of multiparous Holstein cows. J. Dairy Sci. 102, 5191–5520.
Kimura, K., Reinhardt, T.A., Goff, J.P., 2006. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J. Dairy Sci. 89, 2588–2595.
Lean, I.J., Santos, J.E.P., Block, E., Golder, H.M., 2019. Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows: a meta-analysis. J. Dairy Sci. 102, 2103–2133.
Littledike, E.T., Horst, R., 1982. Vitamin D3 toxicity in dairy cows. J. Dairy Sci. 65, 749–759.
Marks, J., Srai, S.K., Biber, J., Murer, H., Unwin, R.J., Debnam, E.S., 2006. Intestinal phosphate absorption and the effect of vitamin D: a comparison of rats with mice. Exp. Physiol. 91, 531–537.
Martens, H., 2016. Diseases of Dairy Animals: Non-infectious Diseases: Grass Tetany, Reference Module in Food Sciences. Elsevier, pp. 1–7.
Martin-Tereso, J., Martens, H., 2014. Calcium and magnesium physiology and nutrition in relation to the prevention of Milk fever and tetany (dietary Management of Macrominerals in preventing disease). Vet. Clin. North Am. Food Anim. Pract. 30, 643–670.

Megahed, A.A., Hiew, M.V.W.H., El Badawy, S.A., Constable, P.D., 2018. Plasma calcium concentrations are decreased at least 9 hours before parturition in multiparous Holstein-Friesian cattle in a herd fed an acidogenic diet during late gestation. J. Dairy Sci. 101, 1365–1378.

Meyer-Binzegger, M., Ollagnier, C., Eggerschwiler, L., Bühler, K., Meylan, M., Schlegel, P., 2021. Potential of a rumen bolus containing 1,25-dihydroxyvitamin D3 glycosides for the prevention of hypocalcemia in primiparous and multiparous dairy cows. Animal accepted In press.

Miltenburg, C.L., Duffield, T.F., Bienzle, D., Scholtz, E.L., LeBlanc, S.J., 2016. Randomized clinical trial of a calcium supplement for improvement of health in dairy cows in early lactation. J. Dairy Sci. 99, 6550–6562.

Mulligan, F., O'Grady, L., Rice, D., Doherty, M., 2006. Production diseases of the transition cow: milk fever and subclinical hypocalcaemia. Ir. Vet. J. 59, 697–702.

Napoli, J., Reeve, L., Eisman, J., Schnoes, H., DeLuca, H., 1977. *Solanum glaucophyllum* as source of 1,25 dihydroxyvitamin D 3. J. Biol. Chem. 252, 2580–2583.

Oberson, J.-L., Probst, S., Schlegel, P., 2019. Magnesium absorption as influenced by the rumen passage kinetics in lactating dairy cows fed modified levels of fibre and protein. Animal 13, 1412–1420.

Oetzel, G., 2011. Diseases of dairy animals: non-infectious diseases: milk fever. In: Encyclopedia of Dairy Sciences: Second Edition, pp. 239–245.

Perruchoud, T., Maeschli, A., Bachmann, H., Walkenhorst, M., Schüpbach, G., Mevissen, M., Zanolari, P., 2017. Diagnose, Therapie und prophylaktische Massnahmen der Gebärparese beim Rind: Ergebnisse der online-Umfrage bei Schweizer Tierärzten. Schweiz. Arch. Tierheilkd. Band 159, 335–343.

Reinhardt, T.A., Horst, R.L., Goff, J.P., 1988. Calcium, phosphorus, and magnesium homeostasis in ruminants. Vet. Clin. North Am. Food Anim. Pract. 4, 331–350.

Reinhardt, T.A., Lippolis, J.D., McCluskey, B.J., Goff, J.P., Horst, R.L., 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188, 122–124.

Rérat, M., Schlegel, P., 2014. Effect of dietary potassium and anionic salts on acid–base and mineral status in periparturient cows. J. Anim. Physiol. Anim. Nutr. 98, 458–466.

Roche, J.R., Heiser, A., Crookenden, M.A., Burke, C.R., Rutner, S.A., Kuhn-Sherlock, B., Phyn, C.V.C., 2018. The effect of feeding synthetic zeolite a prepartum on indices of mineral and metabolic status, milk production and reproduction in grazing dairy cows. J. Dairy Sci. 101 (Suppl. 2), 175.

Rodriguez, E.M., Aris, A., Bach, A., 2017. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. J. Dairy Sci. 100, 7427–7434.

Roux, R., Davicco, M.-J., Carrillo, B.J., Barlet, J.-P., 1979. *Solanum glaucophyllum* in pregnant cows. Effect on colostrum mineral composition and plasma calcium and phosphorus levels in dams and newborn calves. Ann. Biol. Anim. Biochim. Biophys. 19, 91–101.

Santos, J.E.P., Lean, I.J., Golder, H., Block, E., 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J. Dairy Sci. 102, 2134–2154.

Seifi, H.A., Kia, S., 2018. Subclinical hypocalcemia in dairy cows: pathophysiology, consequences and monitoring. Iran. J. Vet. Sci. Technol. 9, 1–15.

Starič, J., Hodnik, J.J., 2021. Biochemical bone markers during the transition period are not influenced by parenteral treatment with a high dose of cholecalciferol but can predict milk fever in dairy cows. Front. Vet. Sci. 7, 591324.

Thilsing-Hansen, T., Jørgensen, R.J., Østergaard, S., 2002. Milk fever control principles: a review. Acta Vet. Scand. 43, 1–19.

van de Graaf, S., Hoenderop, J., Bindels, R., 2006. Regulation of TRPV5 and TRPV6 by associated proteins. Am. J. Physiol. Ren. Physiol. 290, 1295–1302.

Venjakob, P., Borchardt, S., Heuwieser, W., 2017. Hypocalcemia—cow-level prevalence and preventive strategies in German dairy herds. J. Dairy Sci. 100, 9258–9266.

Vieira-Neto, A., Lima, I.R.P., Lopes Jr., F., Lopera, C., Zimpel, R., Sinedino, L.D.P., Jeong, K.C., Galvão, K., Thatcher, W.W., Nelson, C.D., Santos, J.E.P., 2017. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. J. Dairy Sci. 100, 1–19.

Vieira-Neto, A., Negro, G., Zimpel, R., Poindexter, M., Lopes Jr., F., Thatcher, W.W., Nelson, C.D., Santos, J.E.P., 2021. Effects of injectable calcitriol on mineral metabolism and postpartum health and performance in dairy cows. J. Dairy Sci. 104, 683–701.

Wilms, J., Wang, G., Doelman, J., Jacobs, M., Martin-Tereso, J., 2019. Intravenous calcium infusion in a calving protocol disrupts calcium homeostasis compared with an oral calcium supplement. J. Dairy Sci. 102, 6056–6064.